Lab Session 24-06 to 26-07

09 August 2015 00:01

Lab Session 26.07.2015

Glycerol stocks are made for T5 promoter.

Specification: Culture: 800 uL

Autoclaved 100% glycerol: 200 uL

Plasmid isolation was done for:

- NrfA Bio-brick (Gene)
- CysI Bio-brick (Gene)
- T5 Promoter

Nano-dropic Readings:

	Concentration
T5	50
NrfA	60
CysI	60

Conclusion:

Concentration obtained is extremely low.

Gel Run was done for:

T5 promoter (Digested: Sample 2).

Conclusion:

Singly digested band was obtained. (Bottom Most). Undigested bands were also obtained.

Results obtained are weird and we don't have much of an explanation of what exactly happened.

We were uncertain about the activity of the restriction enzymes. Thus, to check their activity, the enzymes were set for digesting the NosZ gene.

- EcorI (1 , 2)
- SpeI (3 , 4)
- XbaI (5)

Result:

The enzymes showed negative result.

Lab Session 25.07.2015

Plasmid isolation was done for the following bio bricks:

- T5 Promoter (Two samples)
- CysI Bio-brick
- NrfA Bio-brick

Nano-dropic readings:

Isolated Sample	Concentration	260/280	260/230
T5 2	125.9	1.95	2.29
T5 1	55.3	1.98	1.7
CysI	49.2	1.96	2.66
NrfA	55.5	1.94	1.58

Lab Session 24.07.2015

We revived the promoters:

Took 10 ul of each

- T5 promoter -vial 1(K 17 plate 1) Bb_k592008

- IPTG inducible -vial 2(H 5 plate 4)Bb_r0011

And revived them in : 50 mg chloramphenicol

Change of Strategy

Till this point, we were using the promoter:

Anderson J23119.

Unfortunately, it is the strongest promoter of the Anderson family, but it acts effectively only for plasmids up to 700 Base Pairs.

On the other hand, the plasmids we were using , i.e. NrfA, SqR and CysI have base pairs in the range: 1.5K-1.7K.

Basically, we were proceeding with the wrong promoter and hence were not getting any positive result with the plasmids isolated.

Consequently, we were giving alternative thoughts about the promoters to be used. We came up with the following suitable promoters:

- T5 promoter -vial 1(K 17 plate 1) Bb_k592008

- IPTG inducible -vial 2(H 5 plate 4)Bb_r0011

Lab Session 13.07.2015

Samples of NrfA , Sqr, PelB were pelleted out.

Specifications (Centrifuge):
- Tx- 400
- speed : 8000
- accn/decn : max (9)
- time : 10 min

PelB plasmid was isolated, using a different technique for better consequence.

The procedure followed:

- Pelleted PelB were re-suspended (250 uL P1 buffer)
- It was transferred to micro-centrifuge tube
- This done, it was mixed with 250 uL buffer P2 (for less than 3 minutes)
- Added to 350uL buffer N3 mix

- Centrifuged for 10 minutes (13000 rpm)
- It was kept aside for 30 - 60 sec
- The flow-through was discarded.
- It was again centrifuged for 1 minute
- It was placed in 1.5 ml micro-centrifuge tube
- This done, 60 uL EB buffer was added.
- The solution was kept aside for for 5 minute.
- Finally, it was centrifuged for 1 minute.
- It was stored at 253 K.

Conclusion:

Nano-dropic Reading:

Concentration of PelB: 50.

Since concentration is very low, it has to be isolated again.

At this point of time, we started thinking about the laggings in the strategy.

Proteins were not getting folded in the first place itself. The protein for NrfA gene requires Heme for getting folded, and since we had no source for Heme, the protein couldn't get folded.

However, in case of yeast, there is no need to supply Heme externally. Consequently, we started thinking about using Yeast for all Heme related genes.

Lab Session 11.07.2015: PCR

We planned for PCR today.

CysI Primers and NosZ Primers are available.

The details of the primers:

CysI Forward Primer : ATG TCT AGA ATG TAC GTA TAC GAC GAG TAC GAC

CysI Reverse Primer : ATG ATG CAT TTA ATG ATT CGC TGC ATA TAC GCG

NosZ Forward Primer : ATG TCTAGA ATG AGC GAC GAC ACG AAA AGC

NosZ Reverse Primer : ATG ATG CAT TCA AGC CTT TTC CAC CAG CAT

The primers were revived.

Procedure followed for PCR:

PCR for First time: (Using Diluted Templates)

- Concentrated Primer (Concentration: 100 pmol/uL) were made initially:

Primer	Water Added (uL)
CysI (Forward)	532
CysI (Reverse)	491
NosZ (Forward)	444
NosZ (Reverse)	409

- This done, the primers were diluted (Concentration : 10pmol/uL)

Concentrated Primer: 2 uL
Water: 18uL

- Dilution of CysI:

Initial Conc. Of CysI : 125 ng/uL
Volume of CysI to be added: 8uL

Final Conc. Of CysI : 50ng/uL
Final Volume : 20uL

- Dilution of NosZ

Initial Conc. Of NosZ : 325 ng/uL
Volume of NosZ to be added : 3.07 uL

Final Conc. Of NosZ : 50ng/uL
Final Volume : 20uL

- PCR for CysI (Two samples C1 and C2):

	Amount (uL)
Master Mix	5
Water	2
Forward Primer	1
Reverse Primer	1
Template (Diluted)	1

Temperature set up for PCR:

C1: 328 K
C2 : 330.5 K

- PCR for NosZ (Two samples N1 and N2):

	Amount (uL)
Master Mix	5
Water	2
Forward Primer	1
Reverse Primer	1
Template (Diluted)	1

Temperature set up for PCR:

N1: 330.5 K
N2: 333 K

- Consequence:

Things went wrong. Primers might have been converted into dimers.

PCR for Second time: (using Concentrated Samples):

- PCR for CysI (Two samples: C1 and C2):

	Amount (uL)
Master Mix	5
Water	0.2
Forward Primer	1
Reverse Primer	1
Template (Concentrated)	2.8

Temperature:

C1: 331 K
C2: 333 K

- PCR for NosZ (Two samples: N1 and N2):

	Amount (uL)
Master Mix	5
Water	2
Forward Primer	1
Reverse Primer	1
Template (Concentrated)	1

Temperature:

N1: 334 K
N2: 336 K

- Consequence: No definite result.

Lab Session 06.07.2015

We resumed our lab work once again. There was a big gap in between, where we did extensive research over the net. A major step taken was including NosZ gene in our NO_x reduction strategy.

We concentrated mainly on:

NosZ (in LB) :

- Streaking
- Spreading
- Inoculation

Concentration: 125

Lab Session 24.06.2015: Characterization

It was a big day for us! For the very first time, we started with the characterization thing. Characterization of the NrfA plasmid was the basic goal for today's lab.

For characterizing, the following materials were used:

- Nessler's Reagent
- Culture
- Inoculant
- Pipe

The procedure followed for characterization was:

1) Firstly we took the inoculant (NrfA Plasmid) in a small test tube and added a few drops of Nessler's reagent using pipette.

Consequence: Initially, there was no change in color. After waiting for some more time, there was a little change in color.

2) In another test tube, we added ammonia, followed by adding a few drops of Nessler's reagent.

Consequence: A precipitate was obtained.

3) Now we mixed the 2 solutions and added some more of Nessler's reagent.

Consequence: Precipitate was obtained.

4)Now, we took 2 test tubes with the following compositions:

- Inoculant (1 mL) + Nessler's Reagent(1mL)

  - No Precipitate was obtained.

- Ammonia (1 mL) + Nessler's Reagent (1 mL) + Inoculant (1mL)

  - Precipitate was obtained in large amount.

Once we were done with the initials, we finally took the following composition:

Inoculant (1 mL) + Ammonia (100 uL)+ Nessler's Reagent (1 mL)

All these done, we are finally ready to start.

For maintaining the perfect reaction conditions, we shall be doing the experiment inside an ice box.

- We took conc. Nitric Acid in a flask.
- Now we put the Nitric acid (3mL) in the flask containing ice.
- We waited for the gas to reach the other flask.

Consequence:

However, the gas didn't reach the other flask.

So, we planned of doing it again the that day. The reaction wasn't as dangerous as we were expecting. Therefore, we took lesser precautions.

Second Attempt:

- Changes done:
  - Copper pellets used: 4 (instead of 1)
  - Nitric Acid used: 6 mL (instead of 3 mL)
- Initially, no gas was transferring. However, this time, we kept patience.
- The flask was taken out of the ice box and stirred a little.
- After waiting for around 5 minutes, the reaction finally proceeded. NO_2 was going in the other flask.
- In the meantime, we added Nessler's reagent in the innoculant. This done, we changed the culture without Nessler's reagent.
- Finally, the gas was coming. Then, we sucked the gas from one flask to another flask.
- The flask was closed tightly. Then we took a little culture in a test tube and added a some Nessler's Reagent.
- However, there were no immediate results. We kept waiting for some more time.
- Color of the media started changing.. It seems thatNO_2 was dissolving in the media.
- After waiting for around 10 minutes, we added Nessler's reagent and finally we got a precipitate.

Consequence:

We were doubtful regarding the results obtained. So, we are planning to redo the experiment for confirmatory results.